MP74: Development of a Practical Animal Model for Human Spermatogonial Stem Cell Transplantation

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INTRODUCTION

We sought to develop a cadaveric animal model simulating testicular cell transplantation (TCT) as technical preparation for its implementation in humans. We attempted to anticipate the practical and logistical obstacles to TCT and test methods to quantify its success.

We assessed model species, surgeon and assistant positions, needle size, injection rate, injection site, injection volume, sonographic contrast (Optison™) concentration, cell concentration, and post-injection histologic analysis methods.

RESULTS

Figure 1. Testicular cells after enzymatic digestion, fluorescent membrane labeling and fixation viewed using phase contrast (A) and under fluorescence (B).

Figure 2. A: Diagram of Surgeon-Assistant Position: Assistant on patient’s left, with US and surgeon on right. B: Best Procedure: Assistant steadies the testes posteriorly using his right hand. Surgeon identifies rete testis using high-resolution US. Surgeon advances the needle with left hand under sonographic guidance (probe in right hand) through anterior surface of testis to cannulate rete testis. Assistant injects cell suspension with left hand. C: US image shows the needle (arrow) in the rete testis (2 arrows). D: Quantifying success: US image showing the distance travelled (dotted line) by the sonographic contrast from rete testis toward tunica albuginea following injection.

Figure 3. Dissected testes after injection with donor testicular cells mixed with methylene blue in various regions.

Single Cell Suspension of Testicular Cells

“Donor” cells were obtained from testicles of dogs between the ages of 2 months and 3 years of age undergoing routine castration. We bisected the testicles at the midline and obtained testicular tissue. The testicular tissue was then manually separated with a razor blade and digested with collagenase followed by digestion with trypsin and DNase I. The cell suspension was filtered and cells were labeled with the PKH26 Red Fluorescent Cell Linker Kit (Sigma-Aldrich) before being fixed in 2% paraformaldehyde.

Injection of Donor Cells into Recipient Testicles

Fixed cells were added to 20% sonographic contrast (Optison™) were injected using a 19-gauge, 2-inch needle on a 3cc syringe into adult testes under ultrasound (US) guidance. In some cases, methylene blue was added to the cell solution to visualize gross distribution of cells. The assistant was positioned on patient’s left side, with the surgeon positioned on the right and the US to the left of the surgeon (Figure 2).

Histologic Analysis of Cell Distribution

After injection, testicles were immersed in 10% formalin for 24 hours to fix the tissue. Serial sections were taken from the fixed testes and then embedded in paraffin. Microscopic sections were made from the paraffin blocks and injected cell distribution were visualized under a fluorescent microscope.

METHODS

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REFERENCES


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